UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Marketing Service

PROCEDURE FOR DETERMINING FAT ACIDITY IN GRAIN
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I - General Method

The following method may be applied to all cereal grains.

(1) Obtain a representative sample of about 50 grams of the grain (100 grams in the case of corn) by hand quartering or by use of a mechanical sampling device.

(2) Grind the sample as finely as possible with a suitable mill, making sure that the bran or seed coat does not separate from the rest of the kernel and remain in the mill. For best results it is recommended that the grain be ground to such a degree of fineness that at least 90 percent of the ground material will pass through a 40-mesh sieve. A somewhat coarser grind, however, will not materially affect the results.

In case the sample is too moist for proper grinding it may be partially dried by heat prior to grinding. Grain may be heated to temperatures as high as 100° C. (212° F.) without appreciably affecting its fat acidity, provided that such heating is continued only long enough to reduce the moisture to a suitable level for grinding.

(3) Extract a 10-gram portion of the well-mixed ground sample (weighed to an accuracy of ± 0.01 gram) with petroleum ether for approximately 16 hours in a Soxhlet or other suitable type of extractor. Double thickness paper thimbles or Alundum R.A. 360 thimbles should be used to hold the samples.

IT IS IMPORTANT THAT THE EXTRACTION BE STARTED IMMEDIATELY AFTER THE SAMPLE IS GROUND, AS THE FAT ACIDITY IN GROUND GRAIN INCREASES AT A RAPID RATE. IN NO CASE MAY THE GROUND SAMPLE BE ALLOWED TO REMAIN OVERNIGHT BEFORE EXTRACTING.

(4) Remove the solvent from the extract completely by evaporation on a steam bath.

(5) Dissolve the extract in the extraction flask with 50 ml. of a 1:1 solution (by volume) of benzene and ethyl alcohol, the mixture containing 0.02 percent of phenolphthalein. Titrate the dissolved extract with carbonate-free standard alkali to a distinct pink color, or in the case of yellow solutions, to an orange-pink color. (See Section III on color standards for titration end-points.) A blank titration is made on 50 ml. of the benzene-alcohol mixture and this value is subtracted from the titration value of the sample.

In the case of grain having high fat-acidity values, emulsions are sometimes formed during the titration, partially masking the end point. In this event an additional 50 ml. of the alcohol-benzene mixture may be added when the emulsion appears, thus insuring a clear solution for titration. The blank titration in such cases, of course, must be double that determined on a single 50 cc. portion of solvent.

It is convenient to use exactly 0.0178 normal potassium hydroxide for titration in order to simplify calculations. One ml. of this solution contains 1 mg. of potassium hydroxide.

(6) Report fat acidity as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids from 100 grams of grain on a dry-matter basis.

Fat acidity = 10 x (titration minus blank), (assuming 0.0178 normal potassium hydroxide was used). Calculate to a dry-matter basis.

Illustration.- If the titration value of the extract = 2.17 ml. of 0.0178N. KOH,
the titration value of the blank = 0.42 ml. of 0.0178N. KOH,
and the moisture content of the sample = 12.1 percent;
then the fat-acidity value would be $\frac{10 \times (2.17 - 0.42)}{1.000 - 0.121} = 19.9$

II. Rapid Method for Corn

The following rapid method for determining the fat acidity of corn is useful when prompt results are desired. With this method results may be obtained in about 45 minutes, as opposed to 16 hours by the General Method. The total labor involved in making the determination, however, is not less than for the General Method.

(1) Obtain a representative sample of about 100 grams and grind according to the instructions in (1) and (2) under General Method.

SINCE THE FAT ACIDITY OF GROUND CORN INCREASES RAPIDLY ON STANDING, IT IS IMPORTANT THAT THE DETERMINATION BE MADE IMMEDIATELY AFTER THE SAMPLE IS GROUND. IN NO CASE MAY THE GROUND SAMPLE BE ALLOWED TO REMAIN OVERNIGHT BEFORE PROCEEDING WITH THE DETERMINATION.

(2) Weigh out a 20-gram portion of the well-mixed meal to an accuracy of ± 0.01 gram, and transfer to a 100 ml. glass-stoppered flask or bottle.

(3) Add exactly 50 ml. of benzene to each flask, insert the stopper, shake a few seconds to saturate the air in the flask with benzene vapor, momentarily loosen the stopper to release pressure, and replace the stopper.

(4) Shake the flask for 30 minutes using a mechanical shaking device, or shake by hand at frequent intervals for 45 minutes.

(5) Tilt the flask at such an angle that settling will take place in a way that will make decantation easy. Allow the flask to rest in this position at least 3 minutes.

(6) Carefully decant as much of the liquid as possible into a 15 cm. folded filter paper inserted in an 8 cm. glass funnel. Cover the funnel with a glass disk or Petri dish to minimize evaporation. Collect the filtrate in a 25 ml. volumetric flask or an accurately calibrated 25 ml. graduated cylinder of the type used with the Brown-Duvel moisture-testing apparatus.

(7) When exactly 25 ml. of the filtrate has been collected, transfer the filtrate to a 250 ml. Florence flask or extraction flask.

(8) Refill the volumetric flask or cylinder to the 25 ml. mark with 95 percent ethyl alcohol containing 0.04 percent phenolphthalein and transfer to the flask containing the benzene extract.

(9) Titrate the extract with carbonate-free 0.0178 normal potassium hydroxide (See Item (5) under General Method) to a distinct pink color in the case of white corn, or to an orange-pink for yellow corn. (See Section III on color standards for titration end-points.) In the case of high-acidity corn an emulsion may be formed during the titration. The emulsion may be dispelled by the addition of 25 ml. each of benzene and of ethyl alcohol containing 0.04 percent phenolphthalein.

(10) Run a blank titration on a mixture of 25 ml. of benzene and 25 ml. of ethyl alcohol containing 0.04 percent phenolphthalein. For extracts of high-acidity corn to which the additional benzene and alcohol have been added, this blank titration value must be doubled.

(11) Report fat acidity as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids from 100 grams of corn on a dry-matter basis.

Fat acidity = 10 x (titration minus blank.) Calculate to a dry matter basis. (See illustration in Item (6) under General Method.)

III - Color Standards for Titration End Points

Since it is impossible to describe adequately the established titration end points for fat-acidity determinations, color standards have been devised to acquaint the analyst with these end points and thus provide for greater uniformity between laboratories. The end point problem is complicated by the fact that the extracts themselves are of a yellow color that varies greatly in intensity depending on the type of grain and the method used to obtain the extract.

The end point in any case should match a solution containing 5 parts per million of potassium permanganate in distilled water, superimposed upon a solution having the color of the extract being titrated. Such a standard may be attained by adding 2.5 ml. of a 0.01 percent solution of potassium permanganate to each 50 ml. of a solution of potassium dichromate of proper strength to match the original color of the solution being titrated.

The following solutions are required to prepare color standards:

Potassium dichromate, 0.5 percent solution.
Potassium permanganate, 0.01 percent solution (freshly prepared).

To 50 ml. of distilled water in a flask of the same type used for titrating, add dropwise a 0.5 percent solution of potassium dichromate until the solution in the flask matches in color the extract to be titrated. Then add 2.5 ml. of a freshly prepared 0.01 percent solution of potassium permanganate. This final solution may be used as a color standard for the titration end point of the extract. In the case of high-acidity grain when an additional 50 ml. of solvent is added to the extract during titration, add an additional 50 ml. of distilled water and 2.5 ml. of 0.01 percent potassium permanganate to the flask containing the color standard.

It is not possible at this time (1940) to state precise limits in fat-acidity values which definitely indicate degrees of soundness in grain, nor to state fat-acidity values which definitely indicate safe or hazardous storage at a given moisture content. The range in fat-acidity values encountered to date has been from 9 to 247 in the case of corn and from 8 to 110 in the case of wheat. Low fat-acidity values are associated with sound grain, whereas high values are associated with grain which has undergone varying degrees of deterioration.

Further research and experience with the fat-acidity values in commercial lots of grain are needed to determine definitely the relationship between these values and the storage and processing values of the grain.